dent RNA polymerase activity has critically important role in HCV RNA replication and so considered as attractive therapeutic target for designing newer classes of compounds. Present work is to investigate the molecular modelling studies of quinoline derivatives such as Chloroquine, Hydroxychloroquine and Primaquine with HCV RNA polymerase. Results of the modelling studies indicate that the quinoline derivatives strongly interacts with the residues in the primer grip site of the polymerase. Quinoline derivatives were also subjected to HCV RNA subgenomic replicon assay and the results are; HCV RNA synthesis inhibited by Chloroquine at 10.75 mM and Hydroxychloroquine at 6.6 mM. Details of modelling studies will be presented.

doi:10.1016/j.antiviral.2009.02.057

53

Identification and Characterization of Pyrimidinediones as Potent Non-nucleoside Reverse Transcriptase Inhibitors of Hepatitis B virus

Lu Yang*, Todd Parsley, Robert Buckheit Jr.

ImQuest BioSciences, Inc., Frederick, USA

Based on extensive data demonstrating that pyrimidinediones are potent non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTI), we hypothesized that these compounds may inhibit hepatitis B virus (HBV) reverse transcription. We have thus assessed the ability of 68 pyrimidinedione congeners to inhibit HBV replication using a HepG2.2.15 cell culture system and a quantitative PCR assay for the detection of HBV viral DNA in treated cell culture supernatants. Initial screening using a 3-dose concentration scheme resulted in the identification of twelve pyrimidinedione molecules (SJ26, SJ44, SJ46, SJ49, SJ50, SJ53, SJ56, SJ59, SJ68, SJ59, SJ80, and SJ86) which displayed submicromolar EC_{50} values and TC_{50} values greater than 10 mM. Expanded analysis of the anti-HBV activity of these twelve compounds using 9-dose concentration points with half logarithmic dilutions of the compounds demonstrated that 3 of the 12 compounds (SJ59, SJ68, and SJ80) had EC_{50} values less than 1 mM in the expanded full dose response evaluations and two additional compounds (SJ26 and SJ49) had EC_{50} values less than 2.5 mM. Anti-viral activity against a broad range of RNA and DNA viruses indicated that these compounds specifically inhibited the replication of HIV and HBV, supporting the hypothesis that they inhibit reverse transcriptase and providing a rationale for development of a first-in-class NNRTI of HBV and/or HIV–HBV co-infection. Each of these SJ compounds inhibit HIV-1 RT at nanomolar concentration levels. Based on these data, compounds SJ26, SJ49, SJ59, SJ68, and SJ80 are being subjected to further characterization and development as therapeutic agents. Results will be presented detailing the in vitro characterization of these compounds for anti-viral efficacy and toxicity in combination with approved HBV therapeutics (interferon, lamuvidine, and tenofovir), analysis of anti-viral efficacy against lamuvidine-resistant virus, accumulation of intracellular cccDNA and rcDNA, inhibition of HBV reverse transcriptase activity, and toxicity against primary hepatocytes.

doi:10.1016/j.antiviral.2009.02.058

54

Prophylactic Efficacy of Intranasally Administered HSP Nanoparticles for Treating a Lethal SARS-CoV Infection in BALB/c Mice

Dale Barnard[1,*], James Wiley[2], Miles Wandersee[1], Yohichi Kumaki[1], Mark Young[2], Trevor Douglas[2], Allan Harmsen[2]

[1] *Utah State University, Logan, USA;* [2] *Montana State University, Bozeman, USA*

HSP nanoparticle (sHsp-PCN) is a small heat shock protein cage nanoparticle that elicits the formation of inducible bronchoalveolar lymphatic tissue (iBALT) in the lungs. iBALT is a transient tertiary lymphatic tissue that acts like conventional secondary lymphatic tissues in that it generates local primary immune responses—B and T cell protective responses is induced by antigen presentation by APCs. Thus, it might be useful in priming the local immune environment of the lung to prophylactically treat infectious lung disease. A series of experiments were done to determine how to treat a lethal SARS-CoV infection in BALB/c mice with sHsp-PCN. In each experiment, 15 mice were treated intranasally (I.N.) with PSS and10 mice were treated I.N. with sHsp-PCN or with Poly IC:LC at 1 mg/kg, a known inhibitor of death in the lethal SARS-CoV mouse model. When mice were pretreated with sHsp-PCN one time at days −17, −13, −9, −5, −2, all mice survived the infection as did mice treated I.N. with Poly IC:LC (1 mg/kg, qd X +4, +24) with all untreated, infected mice dying. sHsp-PCN was well tolerated in sham infected and infected mice; all mice gained weight. When sHsp-PCN was administered less often prophylactically (qd X1, days −17, −13; qd X1, days −9, −5, −2; qd X1, days −5, −2; qd X1, day −2; or qd X1, day −2, +8 h), the percentage of survivors decreased to 45%, 50%, 50%, 30%, 10%, respectively. As expected, when sHsp-PCN was administered therapeutically (bid X1 at −4 h, +8 h; qd X1 on day 1; qd X2 on days 1, 2; or −4 h, +8 h, days 1, 2), it did not significantly prevent death. In a moderately lethal infection, sHsp-PCN pretreatment appeared to be dose responsive, with 80% survivors at a 5-mg/kg dose, 60% survival with a 2-mg/kg dose, and 40% survivors in mice 0.1 mg/kg; 40% of mice survived receiving PSS. Thus, sHsp-PCN appears to be an effective prophylactic treatment for lethal infections in BALB/c mice induced by mouse-adapted SARS-CoV. The data suggest that this technology might represent a novel way of ameliorating if not preventing pulmonary virus infections in general and should be further pursued.

doi:10.1016/j.antiviral.2009.02.059

55

The Activity of New Cage Compounds Against Avian Influenza Virus (H5N1)

Evgeny Belanov[1,*], Marina Leonova[2], Vladimir Osyanin[2], Pavel Krasnikov[2], Sergey Kuznetsov[2], Andrey Shiraev[2], Evgeny Golovin[2], Olga Serova[1], Nikolay Bormotov[1], Sergei Balakhnin[1], Yurii Klimochkin[2]

[1] *FSRI SRC VB "Vector" Rospotrebnadzor, Novosibirsk, Russia;* [2] *Samara State Technical University, Samara, Russia*

High pathogenic strains of influenza type A (H5N1) virus have been the cause of large-scale death in poultry and death of over 200 humans. Functional derivatives of cage compounds are known as one of perspective classes of organic compounds for search of antiviral agents. During our investigation we have synthesized series of functional derivatives of adamantane: amides, hydrazones, amino, hydroxy, carboxy, carbamoylamino derivatives and wide range of adamantyl substituted oxygen, sulfur and nitrogen containing heterocycles. Antiviral activity of synthesized compounds

was evaluated against influenza A (H5N1) virus. Results of biological tests show that most of synthesized compounds reveal antiviral activity to a greater or lesser extent. Also adamantane containing hydrazide has marked antiviral potency against influenza A virus (H5N1), it inhibits their reproduction at 0.5 mM concentration. Amino derivative, containing adamantylidene unit, suppresses replication of H5N1 virus at 0.7 mM concentration. The presence of great number of high active compounds indicates some common principles of antiviral action of compounds, containing saturated cage moiety. It determines route to new virus inhibitors which block M2 ion channels.

doi:10.1016/j.antiviral.2009.02.060

56

Oseltamivir-resistant Subpopulations of H5N1 Influenza Variants are Genetically Stable and Virulent in Ferrets

Elena A. Govorkova*, Natalia A. Ilyushina, Jennifer L. McClaren, Robert G. Webster

St. Jude Children's Research Hospital, Memphis, USA

H5N1 influenza viruses are emerging as human pathogens, and their high lethality warrants an urgent search for optimal antiviral therapy. While the neuraminidase (NA) inhibitor oseltamivir is currently our first line of defense against a pandemic threat, there is little information about mechanism(s) of emergence of drug-resistance, e.g. co-existence and selective advantage/disadvantage of H5N1 oseltamivir-resistant and sensitive viruses. Here we assessed the biological significance of minor subpopulations of oseltamivir-resistant H5N1 variants carrying H274Y NA mutation in a ferret model. Animals were inoculated with either recombinant wild-type A/Vietnam/1203/04 (clade 1) or A/Turkey/15/06 (clade 2.2) influenza viruses or mixtures containing different ratios of drug-resistant and sensitive variants, and their fitness was evaluated. Sequence analysis of individual clones obtained from nasal washes of ferrets (days 2, 4 and 6 p.i.) revealed genetic stability of the minor subpopulations of resistant and sensitive viruses for both H5N1 viruses. Ferrets inoculated with A/Vietnam/1203/04 oseltamivir-resistant variants were as virulent as sensitive viruses, e.g. animals experienced high fever, weight loss, anorexia, extreme lethargy, severe neurological impairment, and death. Titers of A/Vietnam/1203/04 oseltamivir-resistant and sensitive variants in the upper respiratory tract of ferrets did not differ significantly ($P < 0.05$). A/Turkey/15/06 (H5N1) virus is less pathogenic to ferrets and causes mild, non-lethal disease at infectious dose up to 10^6 EID_{50}/ferret. The animals inoculated with drug-resistant variants of A/Turkey/15/06 (H5N1) virus initially showed milder signs of disease on days 1–3 p.i., but at later time points the pathogenicity pattern was identical for resistant and sensitive variants. Our results suggest that minor subpopulations of oseltamivir-resistant H5N1 variants can be stably maintained in mammalian species and co-exist with drug-sensitive variants.

doi:10.1016/j.antiviral.2009.02.061

57

Inhibition of Influenza Virus Replication: Discovery and Development of Therapeutic Compounds which Suppress Viral RNA Synthesis

Tracy Hartman*, Robert Buckheit Jr.

ImQuest BioSciences, Inc., Frederick, USA

Although influenza outbreaks are usually self-limiting, over 500 million people are infected annually and significant morbidity and mortality result from these infections. Additionally, the potential appearance and spread of a highly virulent pandemic strain of influenza similar to outbreaks of H5N1 viruses in 1918 and 1997 emphasizes the need for new and novel inhibitors of influenza virus. At present, annual vaccinations based on predictions of expected circulating influenza viruses and the use of four approved compounds targeting ion channels and the viral neuraminidase represent the entire arsenal of available therapeutics and preventatives for influenza. Thus, continued development of new and novel antiviral agents for the control of influenza is urgently needed and these agents should be amenable for use in combination with the approved anti-influenza agents. ImQuest BioSciences has worked extensively to develop new anti-infective agents targeting the intracellular replication of RNA viruses, including HIV, hepatitis C virus and influenza/respiratory viruses. We have defined a specific series of compounds with activity against these pathogens that target novel and un-exploited viral replication pathways. These agents act as viral transcriptional inhibitors and appear to specifically interact with cellular microtubule macromolecule transport pathways specific to RNA viral pathogens. We have generated 110 compounds in a series of molecules identified as transcriptional inhibitors of HIV and HCV and these small molecules have been screened for activity against influenza A virus, resulting in the identification of three compounds with EC_{50} values in the ng/mL to low μg/mL range but with somewhat narrow therapeutic indices of approximately 25–50. Additional screening has been performed with a representative panel of influenza A and influenza B viruses, other respiratory viruses, including respiratory syncytial virus, rhinovirus, avian influenza virus and highly pathogenic human H5N1 influenza strains, as well as against other non-HIV RNA viruses in order to evaluate the breadth of activity and specificity of the active agents.

doi:10.1016/j.antiviral.2009.02.062

58

Combinations of 5-Iodo-4′-thio-2′-deoxyuridine and ST-246 or CMX001 Synergistically Inhibit Orthopoxvirus Replication In Vitro

Kathy Keith[1,*], Shalisa Sanders[1], Kamal Tiwari[2], Joseph Maddry[2], John Secrist[2], Robert Jordan[3], Dennis Hruby[3], Randall Lanier[4], George Painter[4], Earl Kern[1], Mark Prichard[1]

[1] *University of Alabama School of Medicine, Birmingham, USA;* [2] *Southern Research Institute, Birmingham, USA;* [3] *SIGA Technologies Inc., Corvallis, USA;* [4] *Chimerix Inc., Research Triangle Park, USA*

The nucleoside analog, 5-iodo-4′-thio-2′-deoxyuridine (4′-thioIDU) has been reported to inhibit the replication of orthopoxviruses both in vitro and in vivo. This highly active compound appeared to be phosphorylated by the vaccinia virus thymidine kinase and acts by a mechanism distinct from that observed with either ST-246 or CMX001. Thus, combinations of 4′-thioIDU with these agents might be expected to result in the synergistic inhibition of viral replication. The evaluation of these